INTRODUCTION

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PURPOSE

SUMMARY OF PROCEDURE

Samples will be dehydrated and pulverized then concentrated down to remove excess water. All samples will be extracted with ethyl acetate and methanol utilizing an accelerated solvent extractor (ASE) and then concentrated to dryness, reconstituted with appropriate solvents, filtered and then analyzed on LC/MS/MS.

INTERFERENCES

There is the potential for cross-contamination of samples if sterile technique is not properly conducted.

SAMPLE HANDLING AND PRESERVATION

1. Cooling samples to 4°C is usually the best approach to sample preservation, supplemented by the appropriate holding time for the analyses requested.

EQUIPMENT NEEDED FOR ANALYSIS

Equipment needed for extraction of honeybee samples may include:

- Nitrile gloves
- Goggles

- Nose and mouth half mask
- 15 mL centrifuge tubes
- Speed Vac (Savant)
- Freeze drier
- Ziploc bags
- Rolling pin
- Vortex
- Centrifuge
- Accelerated Solvent Extractor (ASE)
- Dionex stainless steel 10 mL extraction cells
- Dionex stainless steel 10 mL funnel
- Scale
- Scoopula/spatula
- Measuring spoons
- 60 mL scintillation vials
- Dionex ASE fiber glass filters
- Black masher
- Syringe (10 µL)
- Nitrogen evaporator with water bath
- Vacuum manifold
- Teflon liners for vacuum manifold
- 3 mL luer lock syringes
- 0.25 µm filters
- Glass Cuvettes
- 0.22 µm Spin-X Nylon Centrifuge filter tubes
- 2 mL GC vials

- LC/MS/MS

REAGENTS NEEDED FOR ANALYSIS

Reagents needed for extraction of honeybees samples may include:

- Ethyl acetate
- Methanol
- Ethanol
- Diatomaceous earth
- Alumina
- Thiamethoxam –D3
- Ottawa sand
- Milli-q water

SAMPLE PREPARATION FOR ANALYSIS

Place honeybee samples (either in centrifuge tube or in Ziploc bag) on the freezer drier overnight. This is done to remove any excess water that the sample is retaining. Weigh out 2 g of honeybees, record exact weight per sample and place into a pre-labeled Ziploc bag. Spike with 2 µL of 1000 ppm internal standard, Thiamethoxam-D3. Add about 2 grams of diatomaceous earth to each sample. Crush honeybees in the Ziploc bag with hands and/or rolling pin until everything is a fine powder. Transfer sample to a pre-labeled 15 mL centrifuge tube (both the cap and the tube labeled) using the 10 mL funnel that was supplied from Dionex. Add about 1.5 mL of ethanol to the bag and hand massage all the corners to remove excess bee residue. Pour the ethanol into the 15 mL centrifuge tube with the crushed up honeybees and repeat the ethanol step again for a total of two rinses. Place the 15 mL centrifuge tube on the speed vac overnight to remove the ethanol.

For placing honeybee samples on the ASE, disassemble the 10 mL stainless steel cell. Place a 27-mm disposable glass fiber filter at the bottom end of the cell, push the filter into place so that the PEEK is not covered. Attach the bottom end cap to a 10 mL cell body with the Dionex logo at the top. Add a scoop at ½ teaspoon (~2.5g) of alumina to the bottom of the cell using the corresponding funnel. Carefully transfer the honeybee

sample to the cell utilizing the funnel, black masher and spatula. Compact the sample into the cell with the black masher and fill the empty space in the cell with Ottawa sand. Clean the outer area of the cell with a wipe or brush to remove any unwanted debris so that the cell can seal properly. Hand tighten the top cap to the cell body. Place the cell on the ASE with a corresponding pre-labeled 60 mL scintillation vial in the collection tray.

Method utilized on ASE: Temperature 100°C, heat and static time for 5 minutes each with 2 cycles. Rinse volume 60%, purge at 60 seconds and the solvents are ethyl acetate (EtOAc) and methanol (MeOH) at a 2:1 ratio.

Evaporate sample under a steady stream of nitrogen until ~3 mL remains. Using the vacuum manifold replace all the inlet liners with new clean liners and clean them with a squirt of methanol. Place a corresponding pre-labeled glass cuvette in the holder inside the manifold. Replace the top with the clean liners so that each liner goes into a glass cuvette. On top of the inlets attach a 0.25µm filter disk with a 3 mL luer lock syringe. Vortex the honeybee sample until well mixed for at least 10 seconds adding a little MeOH and EtOAc to the sample if dry. Using a glass Pasteur pipette transfer the sample to the corresponding syringe filter. To the scintillation vial add ~2 mL of MeOH to honeybee sample and vortex for at least 10 seconds and transfer that aliquot to the syringe filter. Repeat this step a three more times, using MeOH, a 1:1 ratio of MeOH:EtOAc and 100% EtOAc. After vortexing add to the syringe filter, the plunger can be utilized to push down solvent. When done remove the glass cuvette from holder and evaporate to dryness under a steady stream of nitrogen and a water bath at 45°C.

Reconstitute in the same glass cuvette with 1 mL of 10% MeOH:H_20. Vortex sample for 30 seconds or until well mixed and transfer approximately 1 mL of extract into a pre-labeled 2 mL Spin-X centrifuge tube with 0.22 µm removable filter with corresponding sample identification. Centrifuge Spin-X Filter tubes for 15 minutes at 13,500rpm and then transfer filtered extract to pre-labeled 2 mL GC vial with corresponding sample identification. Extract is ready to be analyzed on LC/MS/MS, or stored in a freezer environment until analyzed.

SOURCES OF ERROR AND VARIABILITY

• Cross-contamination

PERSONNEL QUALIFICATIONS

Personnel should be trained in sterile technique. A short, hands-on field sampling introduction and training should be required. Personnel should have completed the Lab Safety training, any annual refresher training, and any equipment specific training.

HEALTH & SAFETY

Personnel must be thoroughly acquainted with the potential hazards of the reagents, solvents, equipment and procedures described in this SOP. The current Material Safety Data Sheets for the solvents used in this procedure can be found online at http://jr.chemwatch.net/chemwatch.web/dashboard

Follow general laboratory safety procedures, including wearing closed-toe shoes, goggles, lab coat and gloves when performing this method.

DOCUMENT CONTROL

EFFECTIVE DATE	CHANGES TO THE DOCUMENT	VERSION REVISED	WHO